Bone Engineering: Mimicking the In-vivo Calcium Phosphate-Enriched Environment

Yoke Chin Chai [1,2], Scott J. Roberts[1,2], Jan Schrooten[2,3], Frank P. Luyten[1,2]
Corresponding Author: yokechin.chai@med.kuleuven.be
[1]Laboratory for Skeletal Development and Joint Disorders; [2]Prometheus, Division of Skeletal Tissue Engineering; [3]Department of Metallurgy and Materials Engineering, Katholieke Universiteit Leuven, Leuven, Belgium.

Introduction

The use of calcium phosphate (CaP) based carriers in bone engineering is a promising approach for a local in-vivo enrichment of calcium (Ca^{2+}) and phosphate (P_i) to trigger progenitor/stem cells towards bone formation. The aim of this study was to mimic the Ca^{2+} and P_i ion-enriched environment in-vitro to assess the effect of these ions on the proliferation and osteogenic differentiation of human periosteum derived cells (hPDCs).

Materials and Methods

Culture medium containing 1% Ca^{2+} or P_i (0, 2, 4, 6, 8 and 10 mM) in hepes buffered solutions were added to hPDCs cultures and incubated for 1, 3, 7, 14, 21 and 28 days. Cell proliferation, cell cycle progression, alkaline phosphatase (ALP) activity, expression of osteogenic marker genes (osteocalcin (OCN), osteopontin (OPN), bone morphogenetic protein-2 (BMP-2) and Runx2) and mineralisation were evaluated. Statistical significance was established at $\alpha < 0.05$ by using unpaired student t-test (two-tailed) or one-way ANOVA analysis.

Results

The Ca^{2+} and P_i treatment caused a time- and dose-dependent phenotypic change and aggregation of hPDCs, without any cell death after 28 days of culture. 4 to 8 mM of Ca^{2+} and P_i enhanced cell growth significantly from day 3 till 28 similar to mitogenic effect of osteogenic medium. This effect was supported by cell cycle analysis showing a dose- and time-dependent shift towards cells in synthesis (S) and mitotic (M) phases. However, a significant increase in ALP activity could not be detected with any treatment. With regard to gene expression, for certain conditions both Ca^{2+} and P_i significantly upregulated OCN and BMP-2 genes as early as from day 7. OPN and Runx2 were upregulated significantly by P_i and to a lesser extent by Ca^{2+}. Alizarin red staining confirmed the presence of mineral deposits in P_i, but not in Ca^{2+} treated cultures after 21 days. Scanning electron microscopy revealed matrix vesicle-like structures (~400 nm) and cell-mineral nodules interactions in P_i-treated samples.

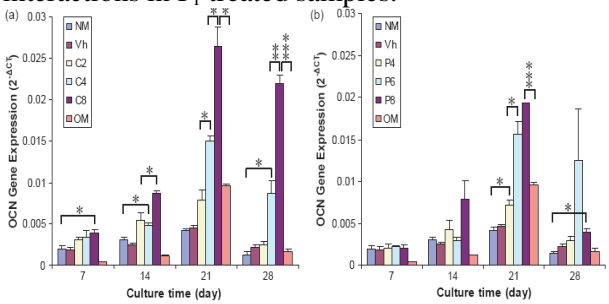

Figure 1: In vitro osteocalcin gene expression of (a) Ca^{2+} and (b) P_i treated hPDCs over the 28 days of treatment. Mean ± S.E.M. (n = 3). Unpaired student t-test (two tailed):*p<0.05, **p<0.01, ***p<0.001.

Discussion and Conclusions

By mimicking the in-vivo CaP-enriched environment in-vitro, we show that Ca^{2+} and P_i induced cell proliferation by regulating cell cycle progression in a dose- and time-dependent manner. This is believed to be essential for achieving a critical cell mass that would initiate bone formation. Dissolved Ca^{2+} and P_i did not induce ALP production, but we observed a dose-dependent upregulation of osteogenic genes expression, important for the regulation of extracellular matrix mineralisation. This study implies a potential method to differentiate stem cells towards the osteogenic lineage for effective bone engineering.

Acknowledgments

This work is funded by the K.U.Leuven IDO 05/009 project and is part of Prometheus, the Leuven Research & Development Division of Skeletal Tissue Engineering of the Katholieke Universiteit Leuven:www.kuleuven.be/prometheus.

Disclosures. No conflict of interest is declared.